Helicobacter pylori Antimicrobial Resistance and the Role of Next-Generation Sequencing

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Abstract: *Helicobacter pylori* infection affects over half of the world's population and is a global health concern because it contributes to chronic and fatal gastrointestinal disorders, including peptic ulcer disease and gastric cancer. The escalating prevalence of antibiotic-resistant strains of *H pylori* necessitates a change in management. The conventional strategy of empiric-based treatments is becoming increasingly ineffective in both adult and pediatric populations; rates of eradication to common first-line regimens remain suboptimal and continue to decline. Culture-based susceptibility testing for *H pylori* has been underutilized and challenging to incorporate into practice. Next-generation sequencing (NGS), which can identify the genetic biomarkers that predict antimicrobial resistance and susceptibility patterns, offers a promising alternative. NGS may enable clinicians to tailor individual treatment regimens and contribute to epidemiologic surveillance across populations. As NGS technology advances and becomes more accessible, its integration into routine clinical practice holds the potential to transform *H pylori* management strategies and improve patient outcomes. This article reviews the literature describing antimicrobial resistance patterns in adult and pediatric practice in the United States and provides practical guidance on the current role of NGS in the management of *H pylori*.

Keywords
Antimicrobial susceptibility, resistance, *Helicobacter pylori*, adult gastroenterology, pediatric gastroenterology, next-generation sequencing

Helicobacter pylori is a widely prevalent organism throughout the world, with an estimated prevalence of approximately 35% in the United States.[1] Eradication of *H pylori* is important given the bacterium's association with pathologic gastrointestinal sequelae, including chronic gastritis, gastric and duodenal ulcers, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma.[2] Antibiotics combined with proton pump inhibitors are the cornerstone of *H pylori* treatment. Overuse and misuse of antibiotics, along with the

bacterium's adaptability, have contributed to the emergence of antibiotic-resistant strains that pose a growing treatment challenge. Antibiotic resistance, in turn, leads to treatment failures and increased clinical complications, underscoring the need for alternative approaches.

Unlike for other common bacterial infections, testing for resistance has not been widely used in the management of *H pylori* in the United States. *H pylori* is a challenging organism to grow in culture from gastric biopsies; consequently, only a few laboratories nationally test for phenotypic resistance, and they are in most cases located far away from the facilities where endoscopy is performed. Molecular testing for the genetic basis underlying the resistant phenotypes is now available as an alternative to culture-based susceptibility profiling, is less prone to the challenges of *H pylori* sample handling for cultures, and is capable of providing more rapid results. Next-generation sequencing (NGS) has emerged as a potentially transformative tool for assessing antibiotic resistance in *H pylori*. NGS enables rapid and comprehensive analysis of the bacterium's genetic material, identifying specific mutations linked to resistance. This technology may allow for personalized treatment regimens based on the unique resistance profiles of individual strains, offering a promising strategy to combat antibiotic resistance and improve outcomes. This article summarizes the latest research on *H pylori* infection in both adult and pediatric practice in the United States and offers practical advice on the emerging role of NGS in current *H pylori* management.

Helicobacter pylori Infection First-line Empiric Treatments

There are several guidelines for the management of *H pylori* infection from which recommendations continue to evolve with new methods for resistance testing and novel treatment regimens. Most relevant for the US adult population are the guidelines put forth by the American College of Gastroenterology and the Maastricht consensus of international *H pylori* experts.[3,4] For pediatrics, joint guidelines for *H pylori* management from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition/North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN/NASPGHAN) were last updated in 2016 and are currently being revised.[5]

There are important differences between the adult and pediatric practice recommendations. For adults, the most recently published 2022 Maastricht VI consensus guidelines recommend a test-and-treat strategy with noninvasive testing for patients with dyspeptic symptoms and the use of eradication therapy whenever detected.[4] Susceptibility testing (molecular or agar culture) prior to prescribing first-line treatment is recommended when possible. When susceptibility is unknown or tests are unavailable, the recommended first-line empiric therapy is bismuth-based quadruple therapy (proton pump inhibitor, bismuth, metronidazole, and tetracycline) for 14 days.[4]

For children, the ESPGHAN/NASPGHAN guidelines recommend against a test-and-treat strategy primarily because there is a lack of evidence supporting a clear benefit of *H pylori* eradication in children.[6] Although testing for *H pylori* is supported in children with certain risk factors (eg, children with gastric or duodenal ulcers, with a first-degree relative with gastric cancer, and with unexplained refractory iron deficiency anemia),[7] literature shows that *H pylori* infection in the absence of peptic ulcer disease is not associated with symptoms, and that eradication does not improve symptoms in children with functional abdominal pain.[7,8] In addition, the benefits of eradication of *H pylori* in pediatric populations is counterbalanced by a potential positive effect of *H pylori* childhood infection, as it may decrease the development of allergic illnesses and inflammatory bowel disease.[9] Moreover, young children may clear *H pylori* over the course of childhood when they are prescribed antibiotics for common pediatric illnesses, making targeted treatment less necessary. Finally, *H pylori* reinfection rates of children are very high, making even successful treatment less likely to be durable.[10] For all these reasons, the treatment of *H pylori* in children warrants careful consideration and shared decision-making with families.

As with Maastricht VI recommendations, current guidelines from ESPGHAN/NASPGHAN recommend testing and treating for *H pylori* among children with chronic idiopathic thrombocytopenic purpura (ITP) to improve platelet counts. However, a more recent meta-analysis failed to show any added benefit of *H pylori* treatment among pediatric ITP patients.[11] As such, future pediatric *H pylori* guidelines may change.

When prescribing treatment regimens, both adult and pediatric guidelines encourage susceptibility testing in specific clinical settings. When testing is not obtained, practitioners are advised to incorporate local susceptibility patterns, and where these data are unavailable, to employ first-line empiric regimens that avoid the use of clarithromycin. However, despite these recommendations, there continues to be high use of clarithromycin among adult and pediatric populations, with clarithromycin prescribed in 45% to 86% of regimens.[12-14] Similar to adult guidelines, in the absence of susceptibility testing, pediatric guidelines recommend bismuth-based quadruple therapy as empiric first-line eradication therapy.[5] Only in cases where resistance patterns are known for clarithromycin and metronidazole is triple therapy including these

Table 1. Single Gene Mutations Responsible for Most Antibiotic Resistance in *Helicobacter pylori* Infection

Antibiotic	Gene	Specific mutations
Amoxicillin	*pbp1a*	Ser414Arg/Asn
Clarithromycin	23S rRNA	A2142G, A2143G, A2142C
Levofloxacin	*gyrA*	A260T (Asn87Ile), T261A, T261G (Asn87Lys), G271A (Asp91Asn), G271T (Asp91Gly)
Metronidazole	*rdxA*	R16HC, M21R/T/V, C19Y, P51L, A67V, C87Y, C140Y, C184Y, G163D/S/V
Rifabutin	*rpoB*	Mutation in codon 525 to 545
Tetracycline	16S rRNA	TCT926-928AAG, T926A/C927A, T926A/T928G, T926C/C927A, T926C/T928G, C927A/T928G

Adapted from Hulten KG et al.[24]
rRNA, ribosomal RNA.

drugs acceptable.[5] Unfortunately, there continues to be low adherence to ESPGHAN/NASPGHAN guidelines among pediatric gastroenterologists.[15]

Prevalence of *Helicobacter pylori* Antibiotic Resistance in the United States

The escalating antibiotic resistance of *H pylori* poses a significant challenge. Over time, *H pylori* has demonstrated an ability to adapt to antibiotic exposure, leading to reduced efficacy of standard treatment regimens. In turn, the widespread and indiscriminate use of antibiotics has accelerated this trend, fostering the selection of resistant strains. In 2017, the World Health Organization identified clarithromycin-resistant *H pylori* among 16 antibiotic-resistant bacteria that pose the greatest threat to human health, designating these *H pylori* strains as high priority in the same tier as vancomycin-resistant enterococci.[16]

Determining precise resistance rates regionally and nationally has been challenging. Studies are limited by small sample sizes, insufficient statistical power, and data heterogeneity secondary to variability in demographics or resistance testing methods. A recent large meta-analysis examining resistance rates over the past 10 years across 2660 samples in the United States demonstrated a high pooled resistance rate to metronidazole (42.1%), levofloxacin (37.6%), and clarithromycin (31.5%).[17] Conversely, the rate of resistance was low to amoxicillin (2.6%), tetracycline (0.87%), and rifabutin (0.17%). Another large multicenter survey of *H pylori* antimicrobial treatment and susceptibility data in the United States from 2017 to 2018 using minimum inhibitory concentration (MIC) susceptibility testing similarly revealed a high national resistance rate for clarithromycin (17.4%), metronidazole (43.6%), and levofloxacin (57.8%) and a low rate of resistance for amoxicillin (6.4%), tetracycline (2.8%), and rifabutin (0%).[18] When compared across US geographic regions (East, Central, and West), clarithromycin resistance ranged from 11.1% in the West compared with 23.2% in the East (P=.03), whereas metronidazole and amoxicillin rates were not statistically different by region. Differences in levofloxacin or tetracycline were unable to be examined across regions given the small sample size.

In the pediatric literature, the data are even scarcer but reveal a similar pattern. Recently, one of the largest pediatric studies in the United States across 2 academic centers in New England using NGS-based techniques demonstrated high rates of resistance to clarithromycin (23.5%), metronidazole (21.9%), and fluoroquinolones (8.4%) and lower rates of resistance to rifabutin (3.6%), amoxicillin (2.4%), and tetracycline (<1%).[19] Unsurprisingly, *H pylori* eradication failure among clarithromycin-resistant and metronidazole-resistant strains was higher among pediatric patients receiving clarithromycin-based and metronidazole-based therapies, respectively.[19]

Additional investigation is needed on the overall prevalence of *H pylori* resistance and eradication rates in the United States in order to achieve higher eradication rates. The current national data are insufficient to assess resistance by ethnicity or geographic variation. In the US meta-analysis discussed previously,[17] there was only a small subset of strains from treatment-naive patients. In most cases, clinical details were lacking as to whether or not the strains were from treatment-experienced patients. By comparison, the international multicenter European

registry on *Helicobacter pylori* management, known as Hp-EuReg, created in 2013, has been prospectively collecting data from more than 70,000 patients throughout 37 countries.[20] With clinical details, and in some cases resistance profiling, as well as outcome data, the information obtained from this cohort has been disseminated to help guide practitioners in this registry to an increase in successful first-line eradication rates from 85% to 93% over the past decade.

Despite the limited quality of data available, rising resistance patterns suggest that selecting an empiric antibiotic regimen without awareness of the local susceptibility is no longer appropriate for *H pylori* management. In particular, clarithromycin and levofloxacin, which had historically been part of standard empiric treatment regimens, are no longer recommended and should only be used in refractory cases or in cases with proven susceptibility.[21] This is particularly important for levofloxacin given the concerns over serious side effects that have resulted in a black box warning for fluoroquinolones from the US Food and Drug Administration.[22]

Next-Generation Sequencing for *Helicobacter pylori*

Resistance Assessment

H pylori is a bacterium adept at quickly gaining resistance to antimicrobials owing to its high mutation frequency from point mutations, a faulty DNA mismatch repair system, and increased recombination under stress states.[23] These factors collectively contribute to a high genetic diversity of *H pylori* populations. Molecular techniques hold potential for delineating the subpopulations of *H pylori* carrying certain specific mutations, which may lead to antimicrobial resistance. A limited number of specific point mutations correlate with most cases of antibiotic resistance, as shown in Table 1.[24] The genetic correlates that accurately predict resistance are best established for clarithromycin (point mutations in 23S ribosomal RNA), levofloxacin (point mutations in the *gyrA* gene), and amoxicillin (mutations in *pbp1a*), as there are just a relatively small number of mutations responsible for essentially all resistance for each of these antimicrobials.[25] In contrast, for antimicrobials that have more complex resistance mechanisms that are multifactorial, additional research is still needed to elucidate all the genotypic variants that predict antibiotic resistance. In particular, the prediction of resistance based on genotype remains challenging for metronidazole, tetracycline, and rifabutin.[24,26] For metronidazole this difficulty stems from the fact that metronidazole resistance is multifaceted, and most metronidazole-resistant *H pylori* strains carry multiple genetic mutations beyond the single gene mutations listed in Table 1. For tetracycline and rifabutin, because of their overall low resistance rates, it is difficult to obtain accurate analysis of the genetic mutations that predict resistance.[26] It is also important to note that nongenomic mechanisms, such as drug efflux pumps and biofilm formation, can also contribute to multidrug resistance in *H pylori* strains.[27]

Molecular DNA sequencing involves the process of identifying the order of nucleotides within a strand of DNA. In 1977, Sanger chain-termination sequencing emerged as the initial technique, but it only allows for sequencing short DNA fragments in series to then consecutively link them into larger whole sequences, which is time-consuming and laborious. Later, shotgun sequencing methods, which randomly fragment a sample of DNA into sequences of varying lengths that are repetitively sequenced in parallel and thereafter aligned linearly with pattern-matching, greatly expanded the speed of sequencing. Now, NGS extends shotgun sequencing still further by making even more and smaller random fragments of a DNA sample that are then sequenced in parallel on a larger scale and aligned. NGS technology provides high throughput analysis, allowing billions of DNA fragments to be sequenced simultaneously, resulting in substantial reductions in time and cost. Although there are various commercially available NGS platforms that vary in technique and read lengths, they all share the same basic principle: DNA is cut into small fragments, amplified, sequenced, and mapped against a known reference genome to identify variants in relevant biomarkers. In 1997, *H pylori* strain 26695 was one of the first bacterial species to have its genome fully sequenced by Sanger sequencing and was demonstrated to have a circular genome of 1,667,867 base pairs with 1590 predicted coding sequences.[28] Over the past 25 years with the emergence of high throughput sequencing NGS technology, there are now more than 2000 *H pylori* genomes recorded in the Pathosystems Resource Integration Center database.[29,30] In addition to whole genome sequencing, NGS may be used to sequence exomes, transcriptomes, and targeted gene regions.[31] Although targeted molecular assays such as polymerase chain reaction or fluorescent in situ hybridization can identify the presence of 1 or a few of these mutations in tissue samples or stool, NGS can evaluate a much broader pattern of mutations in multiple genes of interest simultaneously.

Advantages Over Culture-based Resistance Testing

Given the fastidious nature of *H pylori* isolates, traditional culture-based susceptibility testing is difficult, requiring appropriate biopsy handling, special transport conditions, and a prolonged incubation period under microaerophilic conditions. Consequently, the yield on successful *H pylori*

Table 2. Comparison of NGS With Culture-based Susceptibility Testing

	Culture	Gastric NGS	Stool NGS
Availability[a]	++	+	+
Genotypic-phenotypic correlation	NA	High for clarithromycin and levofloxacin Uncertain for metronidazole	High for clarithromycin and levofloxacin Uncertain for metronidazole
Technical success	Variable, 50%-80%	>90%	>90%
Repeat endoscopy for susceptibility testing	Yes	Yes	No

NA, not applicable; NGS, next-generation sequencing.

[a] ++, available in several US laboratories; +, only available currently from a single US laboratory.

culture is low outside research studies, limiting its use in clinical practice.[32] In one recent study, fewer than 50% of *H pylori*–infected biopsy samples sent to a commercial laboratory were successfully cultured.[19] In contrast, NGS has been shown to be a rapid and sensitive tool to monitor *H pylori* antimicrobial resistance patterns and evolutionary changes. Whereas traditional culture-based susceptibility testing may take 2 weeks for results, the turnaround time for NGS can be as little as 24 to 72 hours, with greater than 90% recovery of DNA suitable to obtain a result.[16] Moreover, NGS can be obtained on fresh or formalin-fixed paraffin-embedded (FFPE) tissue. Thus, repeat biopsy is not required for susceptibility testing in refractory cases; second-line therapy can be based on NGS results from past tissue samples collected for routine diagnostic clinical histopathology. For example, a single-center study retrospectively comparing the rates of *H pylori* eradication with NGS susceptibility testing on FFPE gastric tissue found a statistically higher treatment failure among patients who had received a clarithromycin-containing regimen with NGS clarithromycin resistance (19/34, 55.9%) compared with successes (7/50, 14%; $P<.001$),[33] suggesting the need to find a clarithromycin alternative for these refractory cases.

Even more promising is the advent of stool NGS testing that would provide a noninvasive modality to obtain susceptibility testing. New studies are emerging that show high concordance between stool and gastric biopsy samples. One such study among 70 adult patients with paired stool and gastric tissue NGS testing showed very high concordance rates across all *H pylori* antimicrobials, with *k* coefficient values ranging from 0.88 to 1.00 for clarithromycin, levofloxacin, metronidazole, tetracycline, amoxicillin, and rifabutin.[34] Among 20 pediatric patients, NGS-based stool susceptibility testing was also highly concordant with agar dilution culture-based testing for no resistance (100% agreement), as well as clarithromycin, levofloxacin, and amoxicillin (100%, 67%, and 100% agreement, respectively).[35]

An additional benefit of NGS is the ability to identify hetero-resistance. It has long been recognized that multiple *H pylori* strains may exist within a single stomach.[36] Because hundreds of reads per gene are typically obtained, minority strains can be identified in an individual *H pylori*–infected patient, predicting the emergence of likely treatment failure if resistance is identified in even a small fraction of the overall *H pylori* pool, akin to how sequencing profiles of blood cancers looking at clonal subpopulations helps guide therapy.[37]

With the advancement of molecular-based sequencing techniques over time to monitor increasingly intricate genotypes, the clinical relevance of novel mutations will continue to expand and can allow for retrospective analysis of prior NGS cohorts. In other words, the more knowledge gained of genotypic variants that predict *H pylori* resistance, the more likely it will be to determine more complex resistance patterns, including the mechanisms underlying multidrug resistance. This molecular characterization will also allow researchers to retrospectively monitor changing resistance patterns over time to determine their impact on clinical outcomes, and thereby develop more precise treatment guidelines.

Limitations of Next-Generation Sequencing

Although NGS has many advantages, there are important limitations to consider, including cost and uncertain cost-effectiveness. Currently, a single test costs several hundred dollars and is not reimbursed by most insurance plans. In addition, it should be noted that genotypic resistance as measured by NGS does not always strictly

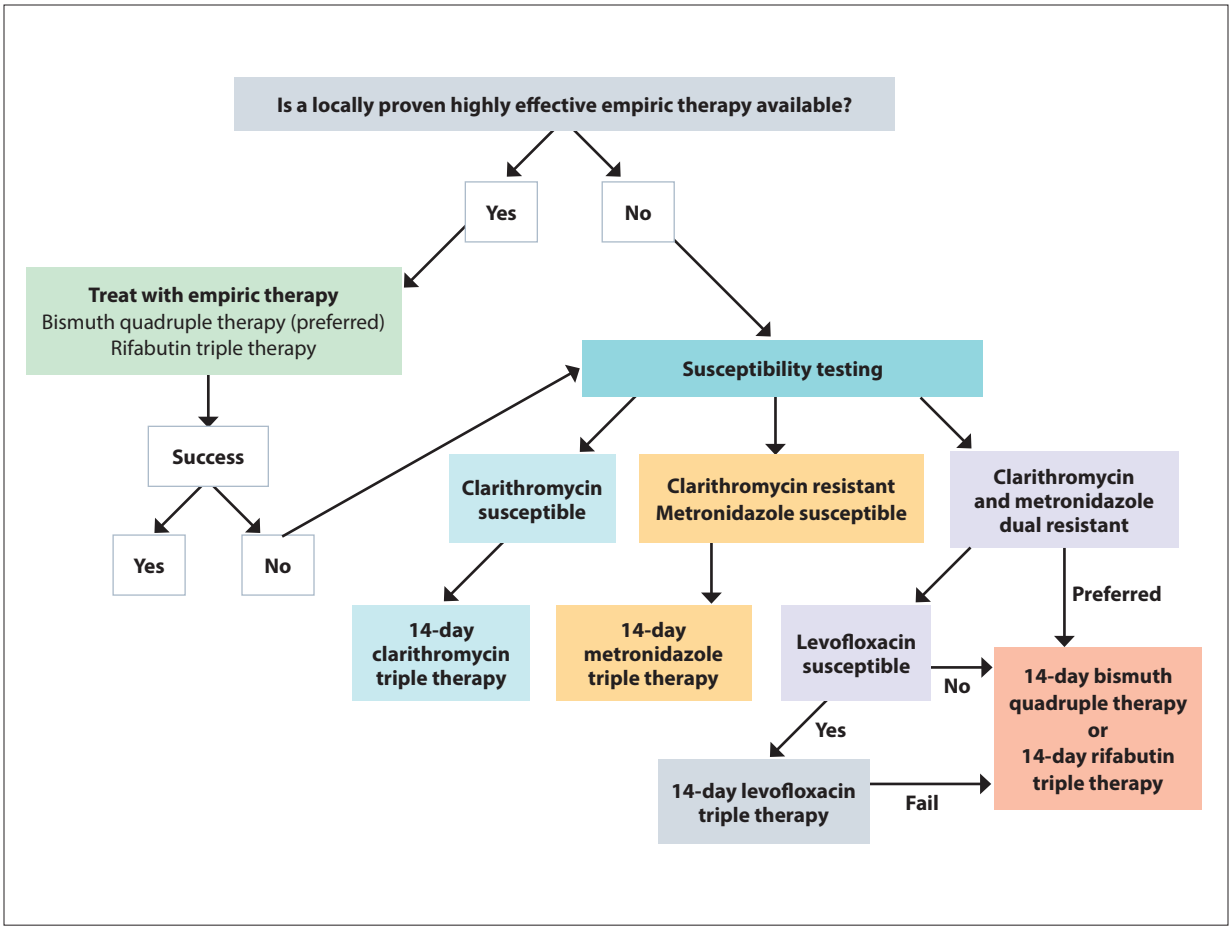

Figure. Proposed algorithm for *Helicobacter pylori* management in adults in the United States. Adapted from Graham DY, Moss SF.[42]

correlate with phenotypic resistance or treatment failure in vivo. Although eradication failure is measured as a binary outcome, therapies contain more than 1 antimicrobial and when NGS predicts resistance to 1 agent, there is still the possibility of successful eradication from the other components in triple or quadruple therapies. The highest genotypic-phenotypic correlations in the literature are observed with clarithromycin, levofloxacin, and amoxicillin, whereas the lowest correlation is with metronidazole.[23,26,35] One recent study compared susceptibility results between MIC agar dilution and NGS obtained from FFPE gastric biopsies among adults aged 18 to 70 years old nationwide and showed high concordance for only clarithromycin (MIC and NGS k = 0.90012) and levofloxacin (MIC vs NGS k = 0.78161).[24] Nonetheless, NGS results in the study reliably (though imperfectly) predicted *H pylori* clinical resistance with high accuracy for clarithromycin (94.1%), amoxicillin (95.9%), and levofloxacin (87.7%). In contrast, there are no reliable data for NGS-based testing to reliably predict resistance to metronidazole with studies showing low concordance rates with agar-based techniques and with overall *H pylori* eradication rates. This is likely because of the complex mechanism of resistance resulting from multiple genetic mutations involved as opposed to single point mutations.[23,26] Additionally, metronidazole resistance can often be overcome in practice with increasing dose or duration of therapy.[38]

When to Consider Susceptibility Testing

A new era of commercially available molecular-based testing should enable appropriate, targeted treatment, leading to improved eradication rates. Susceptibility testing can now be obtained either by culture-based technique, gastric biopsy NGS, or stool NGS (Table 2). A pilot study of an in-house NGS assay for clarithromycin, levofloxacin, and tetracycline susceptibility from gastric biopsies has demonstrated feasibility in routine clinical practice.[39] However, evidence to support the cost-effectiveness of molecular-based testing for all *H*

pylori isolates is still lacking. Perhaps surprisingly, there is still a paucity of high-quality evidence to support the use of any susceptibility testing to improve outcomes in *H pylori* management.[40,41] While awaiting convincing evidence in this regard, a reasonable approach may be a mixed empiric- and susceptibility-based approach. Where there is a high local eradication rate with first-line bismuth-based quadruple empiric therapy, it is reasonable to continue without susceptibility testing. However, where there is a low empiric first-line eradication rate locally or in individual cases of treatment failure to first-line empiric therapy, clinicians should strongly consider proceeding with susceptibility testing to guide appropriate treatment therapy (Figure).[42]

Conclusion

In 2015, the Kyoto global consensus report designated *H pylori* as an infectious disease pathogen. The general principle of antimicrobial stewardship for all infectious disease pathogens as promulgated by the Infectious Diseases Society of America targets a goal cure rate of greater than 90% based on susceptibility-guided treatment regimens.[38] Current eradication rates in the United States fall short of this goal. The conventional strategy of empiric-based treatments is becoming increasingly ineffective with diminishing success rates and escalating resistance rates. There is a greater need for a more tailored and targeted approach to *H pylori* eradication; however, improving *H pylori* management through susceptibility testing has been challenging owing to the difficulty of culturing *H pylori* by traditional agar-based methods. NGS has emerged as a promising tool for assessing antibiotic resistance in *H pylori* infection, allowing for rapid and reliable identification of specific genetic mutations associated with antibiotic resistance. Ultimately, utilization of NGS should lead to a more scientific approach to regimen selection and the promotion of responsible antibiotic stewardship.

Disclosures

Dr Andrews and Dr Herzlinger have no relevant conflicts of interest to disclose. Dr Moss has served as a consultant to Redhill Biopharma and Phathom Pharmaceuticals, which market drugs used to treat H pylori; *he has also received research support from and is a consultant to American Molecular Laboratories, which markets NGS for* H pylori *diagnosis and susceptibility testing.*

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